Induced Pluripotent Stem Cells

What is cellular reprogramming?

Creating Induced Pluripotent Stem Cells (iPSCs)

Cellular differentiation has long been considered a permanent process. That is, once a stem cell has differentiated into a specialized cell, the specialized cell was thought to be trapped in that particular differentiated state. In this manner, specialized cell types were described as 'terminally differentiated', or in other words, specialized cells are incapable of becoming other cell types. This idea that specialized cells are terminally differentiated was challenged by a group of Japanese scientists led by Shinya Yamanaka, who, in 2006, demonstrated that differentiated skin cells could be turned back to a pluripotent embryonic-like state through genetic manipulation of the skin cells in the laboratory. Dr. Yamanaka called these cells induced pluripotent stem cells (iPSCs) because, similar to embryonic stem cells (ESCs), iPSCs are pluripotent – able to differentiate into all cells of the mature organism. However, unlike embryonic stem cells, iPSCs are not derived from the early embryo, but instead created from differentiated cells in a laboratory through a process known as cellular reprogramming. Since the initial discovery in 2006, various types of mouse and human somatic cells have been successfully reprogrammed into iPSCs demonstrating that many differentiated cell types maintain the potential to turn back into unspecialized, pluripotent stem cells.

How were induced pluripotent stem cells discovered?

Discovery of iPSCs

Differentiated cells can be reprogrammed into an induced pluripotent state by the artificial expression of four genes: Oct4, Sox2, Klf4 and c-Myc. Together these four factors (sometimes called the Yamanaka Factors) are naturally expressed in embryonic stem cells, and are not expressed in differentiated cells such as skin cells. As pluripotent stem cells differentiate, they turn off (or silence) the expression of these four genes and begin to express different sets of genes that cause cells to differentiate (or specialize). It is changes in gene expression – turning off pluripotency-related genes and turning on differentiated-related genes – that controls stem cell differentiation into specialized cells. Yamanaka hypothesized that artificially forcing expression of the silenced pluripotency-related genes in differentiated skin cells would turn the specialized skin cells back into pluripotent embryonic-like cells. Scientists in the Yamanaka laboratory began with a list of 24 possible pluripotency-related genes. When all 24 candidate genes were artificially expressed in differentiated skin cells, this caused the skin cells to reprogram into iPSCs. To identify which of the 24 genes were most important for cellular reprogramming, the scientists gradually eliminated genes and tested whether iPSCs could still form. They found that if any one of Oct4, Sox2, Klf4 or c-Myc were not included in the mixture of 24 genes, iPSCs did not form. From these experiments, Yamanaka's team of scientists went on to demonstrate that only four factors: Oct4, Sox2, Klf4 and c-Myc were needed to reprogram mouse or human skin cells into iPSCs.

How are the Yamanaka Factors introduced into cells?

Transgenic expression of the Yamanaka Factors

Because differentiated skin cells do not naturally express Oct4, Sox2, Klf4 and c-Myc, scientists artificially express these factors in differentiated cells. Scientists are able to artificially express genes in cells using transgenic strategies. Transgenics involves the delivery of a sequence of genetic material (a transgene) into cells growing in a petri dish. The transgene typically encodes for a protein that is not actively expressed in the host cell, but when the transgene is delivered into the cell it becomes translated and the functional protein is artificially expressed.

There are a number of different ways to deliver transgenes into cells, including the use of retroviral vectors. Retroviral vectors are modified viruses that deliver foreign genetic material into a cell. Once delivered into the cell, the genetic material becomes integrated into the host cell's genome for long-term expression of the transgene. In the first reprogramming experiments, Yamanaka's group used retroviral vectors to deliver Oct4, Sox2, Klf4 and c-Myc, into skin cells growing in a petri dish. Skin cells that received all four transgenes soon began to artificially express Oct4, Sox2, Klf4 and c-Myc protein. After 2-3 weeks of forced expression of the Yamanaka factors some skin cells underwent genetic changes that caused them to turn into pluripotent embryonic-like stem cells.

Since the first reprogramming experiments, other groups have demonstrated that other non-viral methods for transgene delivery can be used to reprogram differentiated cells into iPSCs. The elimination of viral components from the reprogramming process is a key step in being able to use iPSCs in cellular therapies.

How do scientists know that differentiated cells have been reprogrammed into iPSCs?

Testing for Pluripotency

iPSCs are different from the differentiated cell they started as in many ways. iPSCs can be identified based on morphology (cell shape), genomics (types of genes expressed) and function (ability to differentiate).

Morphology

The first indication that a differentiated cell has been reprogrammed into an iPSCs is a change in morphology of the cells growing in the petri dish. For example, skin cells grow as flattened cells in a dish; however, as they become reprogrammed the iPSCs grow in round clumps known as colonies. The colonies are visible under a microscope, and can be picked using careful techniques in the laboratory. Once colonies are picked, they can be expanded to generate a clonal iPSC population.

Expression of Pluripotency Markers

During reprogramming, the differentiated cells turn off genes that are expressed in a differentiated state, and turn on the expression of genes that are uniquely expressed in an undifferentiated state (pluripotent stem cells). Because these genes are only expressed in pluripotent stem cells and not in other cell types, they are referred to as pluripotency markers. Expression of pluripotency markers is like a molecular signature that lets scientists know that the cells have been reprogrammed to a pluripotent state.

Ability to differentiate into cells of the three germ layers

A key feature of pluripotent stem cells is their ability to differentiate into cell types of the three embryonic germ layers both in vitro (in a petri dish) and in vivo (when injected into an animal). To demonstrate that iPSCs are truly pluripotent, scientists differentiate iPSCs in a dish, and stain the differentiated cells for markers of different cell lineages.

What are the potential applications of iPSCs?

Tool to study human diseases

Currently not enough is known about iPSCs to use them for cellular therapies; however, iPSCs have become useful tools to study causes of human disease in the laboratory. Because iPSCs can be made from a person's own skin, scientists have taken skin samples from patients with a wide range of diseases, and reprogrammed the skin cells into patient-specific iPSCs. Scientists hope that by studying patient-specific iPSCs, they may better understand the underlying causes of the disease, which can potentially lead to more effective therapies.